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OPINION

The heat-shock response in higher plants: a biochemical model

J. J. BURKE & K. A. ORZECH USDA-ARS Plant Stress and Water Conservation Research Unit, Cropping Systems Research Laboratory, Box 215, Route 3, Lubbock, TX 79401, U.S.A.

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Abstract. A compilation of existing data on higher plant responses to heat-shock temperatures has been utilized to produce a biochemically based model of integrated cellular responses to elevated temperatures. This model describes a potential mechanism for the triggering of several biochemical responses to a thermally induced leakage of extracellular or vacuolar ions into the cytoplasm. It seems possible that many of the observed heat-shock responses are involved in the protection of (a) enzymes from inactivation and (b) nucleic acids from cleavage induced by the presence of elevated levels of specific metals.

Key-words: heat shock; model; metals.

Living organisms have developed several endogenous protection systems which provide thermal tolerance. One of these protection systems involves an acquired heat resistance mechanism which is associated with the synthesis and accumulation of specific proteins (HSP). Universally these proteins have been identified by following the incorporation of labelled amino acids into proteins during exposure to elevated temperatures. The heat-shock response has been reviewed both at the protein and molecular levels in numerous articles, reviews and books (Baszczynski, Walden & Atkinson, 1985; Craig, 1986; Key *et al.*, 1982, 1983, 1985; Lindquist, 1986; Nover, 1984).

Early reports of heat-shock proteins in higher plants demonstrated HSP in tobacco and soybean cells grown in solution culture (Barnett *et al.*, 1980), and in soybean seedling tissue (Key, Lin & Chen, 1981). The HSP of soybean were demonstrated by the appearance of 10 new bands on one-dimensional SDS gels; with a more complex pattern on two-dimensional gels (Key *et al.*, 1981). When the tissue was returned to 28 °C after 4 h at 40 °C, there was a progressive decline in the synthesis of HSP and a reappearance of the normal pattern of protein synthesis by 3 to 4 h. To date, the incorporation of radioactive precursors into plant heat-shock proteins has been identified in peas (Hadwiger & Wagoner, 1983; Mansfield & Key, 1987), tomato (Nover & Scharf, 1984; Nover, Scharf & Neumann, 1983), carrot (Pitto *et al.*, 1983), tobacco (Meyer & Chartier, 1983), mung bean (Chen, Kamisaka & Masuda, 1986), barley (Belanger, Brodl & Ho, 1986; Mansfield & Key, 1987), *Tradescantia* (Xiao & Mascarenhas, 1985), *Gladiolus* cormels (Ginzburg & Salomon, 1986, *Lilium longiflorum* (Hong-Qi, Croes & Linskens, 1984), soybean (Key *et al.*, 1981; Mansfield & Key), corn (Baszczynski, Walden & Atkinson, 1983; Bewley, Larson & Papp, 1983; Cooper & Ho, 1983, 1984; Mansfield & Key, 1987), cotton (Burke *et al.*, 1985), wheat (Key *et al.*, 1983; Mansfield & Key, 1987), millet (Key *et al.*, 1983; Mansfield & Key, 1987), sunflowers (Schoffl & Baumann, 1985), sorghum (Ougham & Stoddart, 1986), rice and *Panicum miliaceum* (Mansfield & Key, 1987). The optimal induction temperature for the heat-shock response varies between species, but generally occurs from 10 to 15 °C above the temperature empirically determined for optimal plant growth.

Several inducers of the synthesis of all or specific heat-shock proteins other than elevated temperatures have been identified in both plants and animals. These include developmental controls (Bienz, 1984), metals (Czarnecka *et al.*, 1984), water stress (Heikkila *et al.*, 1984), sulphydryl reagents, calcium ionophores, steroid hormones, chelating agents, pyridoxine, methylene blue, glucosamine, deoxyglucose, and a variety of DNA and RNA viruses (Nover, 1984). Variability exists in the pattern of mRNA and proteins synthesized in response to the magnitude of the temperature shifts (Craig, 1986), chemical inducers (Lindquist, 1986), the degree to which 'normal' protein synthesis is inhibited (Key *et al.*, 1983), and the length of time that maximal HSP synthesis occurs (Baszczynski *et al.*, 1985). The reported cellular localizations of specific HSP in the nucleus (Vincent & Tanguay, 1979), mitochondria (Sinibaldi & Turpen, 1985; Cooper & Ho, 1987), chloroplast (Vierling *et al.*, 1986), endoplasmic reticulum (Baszczynski *et al.*, 1983; Cooper & Ho, 1987), and plasma membrane (Lim *et al.*, 1984; Cooper & Ho, 1987) can also vary depending upon

Correspondence: John J. Burke, USDA-ARS Plant Stress and Water Conservation Research Unit, Cropping Systems Research Laboratory, Box 215, Route 3, Lubbock, TX 79401, U.S.A.

the method of heat-shock protein induction (Lindquist, 1986; Craig, 1986).

One thread common to all of the heat-shock reviews is the inability of investigations to add insight into the question of heat-shock protein function. This paper presents a hypothesis of cellular responses to thermal stress based upon the existing literature, and suggests that some of the proteins associated with the heat-shock response may be associated with the protection of cellular processes from metal inhibition or toxicity. This manuscript is not intended to extensively review the existing literature, but will provide an overview based upon several aspects of the heat shock response in plants. Figure 1 presents a working model of potential cellular responses to high temperature stress.

In this model, elevation of cellular temperature would result in a decreased efficiency of membrane transport systems in the removal of metals and salts from the cytoplasm. This decreased efficiency could be related to a direct effect of temperature on the binding efficiencies of the membrane pumps similar to the temperature-induced Km changes of enzymes in fish and plants (Somero & Low, 1976; Teeri & Peet, 1978). The decline in pump efficiency at either the plasma membrane or tonoplast membrane could result in an alteration of ion transport activity resulting in an elevation of salts (i.e. calcium) and metals (e.g. aluminium, iron, copper and cadmium) in the cytoplasm. Most, if not all, of the other stresses reported to induce the heat-shock response would also affect either the gradient of metals, the energy required to drive the membrane pumps or the transport of salts across the membrane. The leakage of calcium across the membrane would result in the activation of calmodulin [CAM] (Fig. 1, [1]) and, ultimately, enzyme activities associated with calmodulin binding (i.e. membrane ATPases (Fig. 1, [2]), kinases, and phospholipase D (Fig. 1, [3])). The potential activation of phospholipase D could account for the loss of the endoplasmic reticulum's phospholipid membrane during the initial phases of heat-shock (Fig. 1, [4]) (Belanger *et al.*, 1986). The activity of the phospholipase would decline as the calmodulin-activated membrane ATPases removed excess ions from the cytoplasm. Calcium influx into corn roots has previously been reported as a result of cold shock (Zocchi & Hanson, 1982). Zocchi & Hanson (1983) suggested that a Ca^{2+}/H^{+} exchange functions normally to maintain very low Ca^{2+} concentrations, with a Ca^{2+}-ATPase activated only when Ca^{2+} levels rise and calmodulin is activated. A similar activation may occur in response to the heat-shock temperatures.

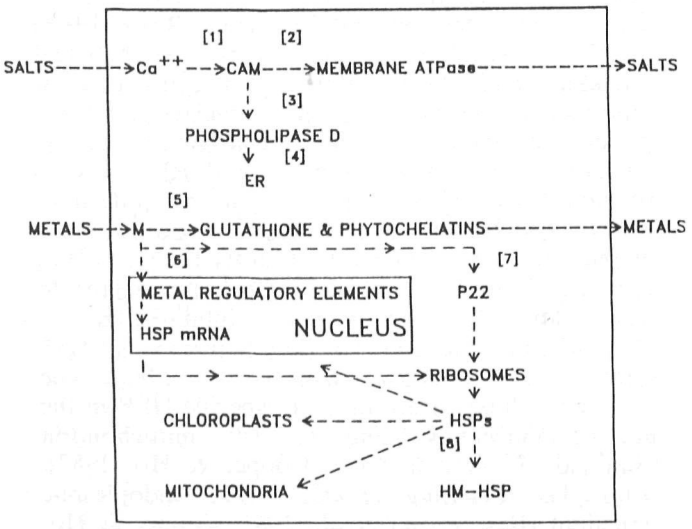

Figure 1. Model of potential cellular responses to high temperature stress. Key reactions [1 to 8] are discussed in the text.

Leakage of metals into cells can be extremely toxic. Cells have evolved mechanisms to aid in scavenging intracellular metals (Rauser, 1981). Glutathione functions as a cellular reductant and has been shown to be involved in removal of cellular toxins (herbicides, metals and so on) (Cherian & Goyer, 1978; Grill, Winnacker & Zenk, 1986). If declining energy levels or direct temperature effects on the kinetic constants of membrane pumps alter the intracellular concentrations of calcium and metals, then increased glutathione concentrations could aid in cellular detoxification (Fig. 1, [5]). To overcome high-temperature-induced kinetic changes in the enzyme responsible for maintaining glutathione in the reduced state (*viz.* glutathione reductase) (Mahan, Burke & Orzech, 1987), elevated levels of glutathione would be required for the maintenance of glutathione reductase activity. The increase in glutathione concentrations reported in high temperature stressed maize (Nieto-Sotelo & Ho, 1986) is consistent with this model. The mechanism responsible for the thermally induced increase in glutathione content has not been identified, however, it may require the synthesis of the enzymes γ-glutamylcysteine synthetase and glutathione synthase. Some metals have been shown to induce the synthesis of linear polymers of glutathione, termed phytochelatins (Grill, Winnacker & Zenk, 1985). These phytochelatins function as metal-sequestering peptides via metal-thiolate co-ordination and have been shown to be induced by a range of metals (Grill *et al.*, 1986). Although specific metal transporters have not been isolated, permeability studies by Gutknecht (1983) suggest that Cd^{2+} transport across membranes is protein mediated and correlates with Cd^{2+}, not $CdCl_2$, concentration.

Concomitant with the initial scavenging of the metals by glutathione and phytochelatins, some of the metals may interact with genes containing similar sequences to the metal ion responsive elements found upstream in metallothionein genes of animals (Fig. 1, [6]) (Serfling *et al.*, 1985). Similar sequences have been reported far upstream in the Gmhsp 17.5-E

(*Glycine max* heat-shock protein, 17.5 kDa) genes (Czarnecka *et al.*, 1985). The degree to which the metals would be available for HSP induction would be related to the level of glutathione, cysteine, and phytochelatins in the cell. This may explain in part the observed changes in the induction temperature of the heat-shock response following several cycles of heat-shock (Key *et al.*, 1985). The reported increases in glutathione content in response to the high temperature stress would provide increased protection from the metals, thereby functionally removing them from HSP induction.

The mechanism regulating the changes in the specificity of ribosomes for the heat-shock mRNA is unknown. The association of a 22 kD (P22) cytoplasmic protein has been reported during the transition to selective mRNA translation (McMullin & Hallberg, 1986). The mechanism of interaction between the P22 and the ribosome, however, remains unknown. It is possible that the binding of the P22 to free ribosomes is associated with metal binding during the thermally-induced leakage of metals into the cell or binding may be related to protein phosphorylation by a calmodulin-activated kinase (Fig. 1, [7]).

Finally, some of the small molecular weight HSP may function in chelation of metals (Fig. 1, [8]). Most metal-chelating proteins that have been identified are low molecular weight proteins whose synthesis is induced by specific metals (Weigel & Jager, 1980). The low-molecular-weight heat-shock proteins have also been shown to be induced by metals, with some specificity between the metal tested and the appearance of mRNA for particular low molecular weight HSP (Czarnecka *et al.*, 1984). These chelating proteins could be protected from proteolysis while binding the metal and become susceptible to proteolysis following removal of the metal. This hypothesis is consistent with the observation that HSP do not accumulate to significant levels following a high-temperature treatment, yet have been shown to accumulate in field-stressed plants where elevation in leaf temperatures is associated with tissue dehydration and water stress (Burke *et al.*, 1985). Water stress can result in tissue dehydration which would increase the cellular ion concentrations, thereby requiring their functional removal via chelation by low molecular weight HSP until the ions could be transported out of the cell or until the stress was alleviated.

In summary, the present authors have presented a working model of possible responses of higher-plant cells to elevated temperatures. Because of the reported induction of heat-shock proteins by metals, and because of the known sensitivity of enzyme systems to temperature changes, the reported cellular responses presented in this model cannot be dismissed based upon the existing heat-shock response literature. Future research on heat-shock responses of higher plants should consider the possibilities addressed in this research model.

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